REMARKS

Claim Rejections - 35 U.S.C. § 132

The Examiner states the Amendment filed July 15, 2003 is rejected to under 35 U.S.C. § 132 because it introduces new matter into the disclosure. 35 U.S.C. § 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: The applicant has amended claims 19-31 and 36-38 to recite "activating a hyperacute rejection in the subject in the absence of gene transfer", or "inhibiting the growth of the tumor in the subject in the absence of gene transfer," or "attacking said tumor in the absence of gene transfer". The specification does not support the phrase "in the absence of gene transfer" (*PTO Paper No.* 14 at pp. 2-3).

Applicants have amended the claims by deleting the recitations "activating a hyperacute rejection in the subject in the absence of gene transfer", or "inhibiting the growth of the tumor in the subject in the absence of gene transfer," or "attacking said tumor in the absence of gene transfer". Additionally, independent claims 19 and 26 have been amended to read on a method of treating a tumor by administering to a human subject xenogeneic cells that cause a hyperacute rejection (see amended claims 19 and 26). Such claims are based upon the discovery by Applicants that delivery of xenogeneic cells to a tumor results in inhibition of tumor growth, and that such a result occurs independently of any gene therapy effects. This idea does not constitute new matter and is described in the specification as filed. Applicants respectfully direct the Examiner's attention to page 18, lines 28-33 to page 19, lines 1-13, wherein Applicants disclose that any xenogeneic cells that will activate the hyperacute rejection will be effective in the invention.

Claim 33 contain by virtue of its dependency, all the limitations of amended claim 32. Although not acceding to the Examiner's rejection, claim 34 has been canceled. Applicants respectfully request this rejection be withdrawn.

Claim Rejections - 35 U.S.C. § 102

Claims 19-39 stand rejection under 35 U.S.C. § 102(a) over Klatzmann et al.

Applicants respectfully traverse this rejection. Applicants are herein submitting for consideration by the Examiner the declaration of Dr. Charles J. Link under 37 C.F.R. 1.131, to overcome Klatzmann et al. *Human Gene Therapy* 9:2585-2594 (November 20, 1998).

Furthermore, Applicants have amended claims 19 and 26 to read on a method of treating a tumor by administering to a human subject xenogeneic cells that cause a hyperacute rejection (see amended independent claims 19 and 26). Such claims are based upon the discovery by Applicants that delivery of xenogeneic cells to a tumor results in inhibition of the tumor, and that such a result occurs independently of any gene therapy effects. This method is supported by the specification. In contrast, Klatzmann describes a method of injecting murine viral producer cells in the vicinity of a tumor in a human subject, to mediate gene transfer of HSV-TK, followed by ganciclovir administration. Applicants invention is different from Klatzmann because the invention described by Applicants does not require gene transfer, does not require or involve treatment with a prodrug and is not limited to vector producer cells as these cells can be xenogeneic cells of any origin except old world monkeys or humans.

Applicants would like to respectfully submit to the Examiner, in view of the amendments made to the claims, that as long as the specification discloses at least one method for making and using the claimed invention and have a reasonable correlation to the entire scope of the claims, then enablement is satisfied. Failure to disclose other methods by which the claimed invention

may be made does not render a claim invalid under 35 U.S.C. § 112. Applicants respectfully submit the enablement requirement is satisfied because the specification discloses at least one method of treating tumors by the administration of xenogeneic cells that is commensurate in scope with the amended claims; therefore, the specification is not required to show it by any other means.

Conclusion

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a response in the above identified application for 1 month from December 25, 2003 to January 25, 2004.

Applicant is a small entity under 37 CFR 1.9 and 1.27. A small entity statement under 37 CFR 1.27 has already been filed in this application.

Please charge Deposit Account No. 26-0084 in the amount of $ 55.00 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to Deposit Account 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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Attorneys of Record

- pw -

Support for the idea that a tumor response can be obtained by injecting xenogeneic cells in or near the tumor site in the absence of gene transfer can be found in the specification as filed. On page 17, line 10, Applicants state: "in animal models, this approach is often not successful even when 50% of the cells are pre-transduced with HSV-TK for a variety of tumor xenografts" ...."a 50% transduction frequency was required to cure 50% of animals"..."therefore high level of gene transfer is required in these model systems." Also, on page 18, line 19 Applicants state: "Quantitative PCR analysis demonstrated less than 1% gene transfer into intraperitoneal tumor biopsies prior to GCV administration"; and on page 18, line 16 says: "the patient with the mixed response demonstrated significant resolution of malignant ascites prior to GCV infusion". The conclusion drawn from these observations is that a favorable tumor response was observed in a patient prior to GCV administration, even in the presence of very low (non-therapeutically effective) gene transfer (~ 1%). Thus, the favorable tumor response observed for that patient cannot be attributed to gene transfer of HSV-TK because a high level of gene transfer (~ 50%) is required to actually observe an antitumor response mediated by gene transfer of HSV-TK. This conclusion is written in page 18, line 28, which states: "Since it is believed that the gene transfer was not necessary to the antitumor reaction, it is believed that any xenogeneic cells which will activate the hyperacute rejection will be effective in the invention." Applicants respectfully request this rejection be withdrawn.

Claim Rejections - 35 U.S.C. § 112, First Paragraph

Claims 19-31 and 36-38 are newly rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner states:

> Applicants' new claims 19-31 and 36-38 introduce new matter into the disclosure of the specification by reciting methods "in the absence of gene transfer" which are not supported by the specification, see also above under 35 U.S.C. § 132.

Id. at 3-4.

Independent claims 19 and 26 have been amended to read on a method of treating a tumor by administering to a human subject xenogeneic cells that cause a hyperacute rejection (see amended claims 19 and 26). Applicants respectfully submit amending the claim to read on "a method of treating a tumor" more clearly defines the invention. This recitation is believed to not introduce new matter or change the direction or scope of the claims because it is supported by the specification as filed and has previously been examined.

Further, Applicants respectfully submit the amendments made to claims 19 and 26 are based upon the discovery by Applicants that delivery of xenogeneic cells to a tumor results in inhibition of the tumor, and that such a result occurs independently of any gene therapy effects. This is supported in the Application as filed. Applicants respectfully direct the Examiner's attention to page 18, lines 28-33 to page 19, lines 1-13, wherein Applicants disclose that any xenogeneic cells that will activate the hyperacute rejection will be effective in the invention. Support for a tumor response can be obtained by injecting xenogeneic cell lines in or near the tumor site in the absence of gene transfer can be found in the specification. On page 17, line 10 Applicants state: "in animal models, this approach is often not successful even when 50% of the cells are pre-transduced with HSV-TK for a variety of tumor xenografts" ...."a 50% transduction frequency was required to cure 50% of animals"..."therefore high level of gene transfer is required in these model systems." Also, on page 18, line 19 says: "Quantitative PCR analysis demonstrated less than 1% gene transfer into intraperitoneal tumor biopsies prior to GCV

administration"; and on page 18, line 16 Applicants state: "the patient with the mixed response demonstrated significant resolution of malignant ascites prior to GCV infusion". The conclusion drawn from these observations is that a favorable tumor response was observed in a patient prior to GCV administration, even in the presence of very low (non-therapeutically effective) gene transfer (~ 1%). Thus, the favorable tumor response observed for that patient cannot be attributed to gene transfer of HSV-TK because high level of gene transfer (~ 50%) is required to actually observe an antitumor response mediated by gene transfer of HSV-TK. See page 18, line 28, which states: "Since it is believed that the gene transfer was not necessary to the antitumor reaction, it is believed that any xenogeneic cells which will activate the hyperacute rejection will be effective in the invention."

One of ordinary skill in the art would be able to determine which cells other than murine cells will be safe and effective. There are many commercial and academic sources of cell lines available. Additionally, one of skill would be able to develop cell lines to use in the present invention; such methods are generally known in the art. The Examples show that the xenogeneic cells were infused into the peritoneal cavity in the vicinity of the tumor using a catheter, but one of skill would be able to determine other methods of presenting xenogeneic cells to the body in order to induce the hyperacute rejection and bystander effect. (See spec pp. 18-19). Furthermore, the dosage levels of xenogeneic cells that have been used are shown in the Examples. Applicants respectfully submit that one of skill would be able to determine a dose level appropriate for the tumor and the patient which is both safe and effective. The remaining claims depend on independent claims 19 and 26; therefore, they contain by virtue of their dependency all the limitations of now patentably distinct independent claims 19 and 26.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 19-34 and 36-38 are newly rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner states:

> Amended or new claims 19-31 and 36-38 recite "in the absence of gene transfer." The specification does not provide support for this limitation or provide a definition of "gene transfer." Therefore, in the absence of any recitation or description of methods "in the absence of gene transfer", or any definition of "in the absence of gene transfer", the metes and bounds of the claims cannot be determined.

Id. at pp. 4-5.

Claims 19-34 and 36-38 have been amended by deleting the recitation "in the absence of gene transfer", thereby alleviating this rejection. Applicants have amended the claims to read on a method of treating a tumor by administering to a human subject cells that cause a hyperacute rejection. Such claims are based upon the discovery by Applicants that delivery of xenogeneic cells to a tumor results in inhibition of the tumor, and that such a result occurs independent of any gene therapy effects.

Next the Examiner states:

> Amended claims 32-34 lack antecedent basis for the limitation, "delivery to the tumor of the murine cell line.

Id. at p. 5.

Claim 32 has been amended by deleting the recitation "administering one or more chemotherapeutic agents to the subject following delivery to the tumor of the murine cell line", thereby rendering this rejection moot. Furthermore, claim 32 has been amended by deleting the recitation "prior to transduction of a HSVtk gene", thereby alleviating this rejection.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	:	LINK, et al.
SERIAL NO	:	09/589,255
FILED	:	June 7, 2000
TITLE	:	METHODS FOR TUMOR TREATMENT USING INFUSION OF XENOGENEIC CELLS TO INDUCE HYPERACUTE REJECTION AND INNOCENT
Grp./A.U.	:	1632
Examiner	:	WEHBE, Anne Marie Sabrina
Conf. No.	:	8671
Docket No.	:	P04091US1

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES OR IN A NAFTA OR WTO MEMBER COUNTRY TO OVERCOME CITED PATENT OR PUBLICATION
(37 CFR 1.131)

Commissioner for Patents
P.O. Box 1450
Mail Stop AF
Alexandria, VA 22313-1450

A. PURPOSE OF DECLARATION

1. This declaration is to establish conception of the invention in this application in the United States, at a time prior to November 20, 1998, that is the publication date of the prior art publication, Klatzmann et al. Human Gene Therapy 9:2585-2595, that has been cited by the Examiner in the office action of September 25, 2003.

2. The person making this declaration is an inventor on the above-identified application.

B. FACTS AND DOCUMENTARY EVIDENCE

3. To establish conception of the invention of this application, the following attached documents are submitted as evidence:

Exhibit A: A letter that evidenced the submission of a clinical protocol sent for recombinant advisory committee (RAC) review in 1995. (1 page).

Exhibit B: A scientific abstract present in the clinical protocol sent for RAC review in 1995 that states "HSV-TK/GCV System induces a bystander effect and an anti-tumor immuno response", thereby recognizing the importance of collateral anti tumor bystander effect mediated by an anti tumor response (1 page).

Exhibit C: A copy of a letter from Dr. Charles Link to Nelson Wivel, Director of RAC, in 1995 that states the effect of peritoneal fluid on the viability of vector producing cells (VPCs) and recognizing that "some toxicity of VPCs will occur on exposure to peritoneal fluid." (5 pages).

Exhibit D: A letter in 1995 from Dr. Charles Link to Dr. Una Ryan, CSO of T-Cells Sciences, and which Dr. Link mentions that "xenogeneic murine vector producer cells are destroyed by human complement mediated lysis against the alpha (1, 3)-galactosyl epitope that is absent from cells old world primates in humans", which introduces the concept that xenogeneic cells of any origin except from old world primates will suffer this hyper acute rejection mediated by

human complement. At that time it was hoped that xenograph rejection was not going to completely affect viability of xenogeneic cells and that due to the absence of blood in the peritoneal cavity, was not going to be strong enough to completely destroy the VPCs allowing them to survive long enough to mediate gene transfer to tumor cells located in the peritoneal cavity. Also in this letter, Dr. Link anticipates that "this strategy would also be attempted for intrahepatic liver tumors. However, since intrahepatic lesions are in contact directly with blood, it was anticipated that compliment mediated destruction of xenogeneic cells would occur more readily under these conditions" and that "this might not allow time for effective gene transfer into tumor cells" thereby anticipating that the method might be carried out in the absence of significant gene transfer (1 page).

4. From these documents, all of which were created and in existence prior to November 20, 1998, it can be seen that the invention in this application was conceived at least by the date of November 20, 1998, which is a date earlier than the publication date of the cited reference. Dates on the documents have been redacted.

5. Specifically, documents of Exhibits A-D show the following concepts from the claims of the application:

   (a) Collateral anti-tumor bystander effect mediated by an anti-tumor immune response (see Exhibit B).

(b) The recognition of the role of hyperacute rejection mediated by alpha (1,3)-galactosyl epitope present in murine viral producer cells and by human serum as one of the mechanisms responsible for triggering local inflammation and possibly mediating an anti tumor response (see Exhibit D).

(c) Xenogeneic murine vector producer cells are destroyed by human compliment mediated lysis against the alpha (1,3)-galactosyl epitope that is absent from cells from old world primates and humans; xenogeneic cells of any origin except from old world primates would suffer this hype acute rejection mediated by human complement (see Exhibit D).

C. DILIGENCE

6. From the time of conception, to a time just prior to the date of the reference, Applicant's diligently moved towards a reduction to practice of the filing of the application identified in the caption of this declaration.

7. Exhibits E-H occurred at a time subsequent to the documents of Exhibits A-D up to June of 1999, the filing of the above-identified application.

8. In 1995 and 1996 development and testing of the murine vector producer cells occurred in the laboratory.

9. Exhibit E (9 pages) shows the start of production of clinical grade materials in 1997 (see Exhibit E: copy for lab notebooks recording the harvest and product lot number). Dates on the documents have been redacted.

10. In August of 1997, treatment of patients started, extending until May of 1999. Exhibit F (24 pages) are copies of data reflecting testing performed to evaluate gene transfer of HSZ-TK into patient tumor biopsies or blood cells in the presence of replication competent retrovirus and some patients, Table 1, reproduced below, shows treatment of patients from a time starting in August of 1997 to May of 1995:

Table1

Patient #	Response	Treatment Date	Death Date
1	Partial	-	05/16/00
2	Mixed	-	01/12/00
3	Progressive	-	12/15/98
4	Progressive	-	05/20/98
5	Progressive	-	08/09/98
6	Minimal	-	01/12/99
7	Progressive	-	01/14/01
8	Complete	-	10/27/02
9	Progressive	-	01/19/99
10	Progressive	-	06/16/00
11	Stable	-	Alive

11. Exhibit G (3 pages) shows that analysis of results obtained on the study led to the observation that VPCs were not recovered from patients seven days after administration suggesting rapid clearance of theses cells after injection in the intraperitoneal cavity. Tumors biopsied on day 14 prior to the administration of GCV showed presence of HSV-TK gene, though at very low levels, approximately 1 percent. Of note, one patient demonstrated a significant resolution of malignant ascites prior to infusion of GCV, implying a role for xenotransplant rejection in the observed anti tumor response. To confirm the

rejection of murine VPCs could have been mediated by anti bodies to the murine alpha (1,3)-galactosyl epitope, anti-alpha GAL antibodies were measured before and after the xenotransplant.

12. Exhibit H (2 pages) shows an abstract to a scientific meeting (gene therapy of cancer VIII) made public in November of 1998, showing results of the above analysis, which showed a 4-16-fold increase in antibody tiders indicating that the process of xenotransplant rejection of murine VPCs induced immune destruction of human cancer.

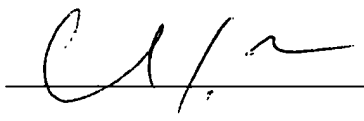
E. DECLARATION

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

F. SIGNATURE(S)

Inventor(s)

Full Name of First Inventor: Charles J. Link, Jr., M.D.

Inventor's Signature: 

Date: 12/30/03

HUMAN GENE THERAPY
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1805

Link–Moorman Protocol

Nelson A. Wivel, M.D.
Director, Office of Recombinant DNA Activities (RAC)
Office of Science Policy and Legislation
National Institutes of Health
Bldg 31 Rm 4B11
Bethesda, MD 20892-0031

Dear Dr. Wivel,

Please find enclosed our protocol entitled "A Phase I Trial of *In Vivo* Gene Therapy with the Herpes Simplex Thymidine Kinase/Ganciclovir System for the Treatment of Refractory or Recurrent Ovarian Cancer" which has been prepared in accordance with the "NIH points to consider for human gene transfer protocols." Also are included three discs with the complete sequence of the LTKOSN.2 retrovirus which we propose to use in this work.

We would like this proposal to be considered at the : RAC meeting. Thank you for your time and effort reviewing this protocol. Please contact me if you require anything further.

Yours sincerely,

Charles J. Link Jr., M.D.
Chief, Laboratory of Molecular Oncology
Associate Director

EXHIBIT
A

I. SCIENTIFIC ABSTRACT

This study will evaluate the safety and efficacy of *in vivo* gene transfer of the Herpes Simplex-thymidine kinase (HStk) gene using PA317/LTKOSN.2 vector producing cells (VPC) in pati nts with recurrent or refractory ovarian cancer. Insertion of the HStk gene into tumor cells confers a sensitivity to the anti-herpes drug ganciclovir (GCV). The HStk/GCV system induces a bystander effect and an anti-tumor immune response. HStk VPC have destroyed intraperitoneal tumors growing in animals. This selective destruction of growing tumors *in situ* is thought to result from the production of toxic GCV metabolites within the tumor. This procedure has resulted in the cure of experimental animals with limited toxicity. Therefore, we propose to apply this technique for the treatment of refractory or relapsed ovarian cancer.

Adult women (≥18 years) with recurrent or refractory ovarian cancer, will be evaluated for the extent and location(s) of their disease before being entered into the study. Patients will have a CT scan and peritonoscopy with biopsy to confirm the diagnosis. During peritonoscopy, eligible patients will have a Tenckhoff catheter placed. HStk VPC will be infused into the peritoneal space. Two weeks later, GCV will be administered at 5 mg/kg/dose IV b.i.d. for 14 days. Patients will only receive one cycle of therapy in this dose escalation protocol. After the completion of the course of GCV, the patient will then be followed at least every 4 weeks for the first 6 months and then at 2 to 6 month intervals. This protocol is related in principle to RAC approved protocols for the treatment of brain tumors in adults and children. This protocol is novel in the site of administration of the HStk VPC and the dose escalation schema.

EXHIBIT

B

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Nelson A. Wivel, M.D.
Director
Office of Recombinant DNA Activities (RAC)
Office of Science Policy and Legislation
National Institutes of Health
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Dear Dr. Wivel,

Enclosed are the responses to the RAC comments on our protocol "A Phase I Trial of *In Vivo* Gene Therapy with the Herpes Simplex Thymidine Kinase/Ganciclovir System for the Treatment of Refractory or Recurrent Ovarian Cancer." We have included the minor changes made in the protocol design. These changes are the result of a meeting on that we conducted with Dr. Parkman, Dr. Straus, and Dr. DeLeon. We have also included the revised consent form as requested by Dr. Zallen.

I certainly understand that the comments from Dr. Rother should be addressed, and we do not consider this request to be "double jeopardy" in the review process. We have addressed the comments by Dr. Rother as requested by Dr. Straus, Dr. Glorioso, and yourself. We elected to perform the experiments suggested by Dr. Rother and include the results of those studies. We wish to again thank the reviewers for their time and helpful comments. Please contact me If there are additional concerns or comments at (515) 241-8787.

Sincerely,

Charles J. Link Jr., M.D.
Associate Director
Chief, Laboratory of Molecular Oncology

EXHIBIT
C

1. RAC Comment: Change the study design to incorporate earlier peritonoscopies to access gene transduction before the administration of ganciclovir. Patients will have an initial peritonoscopy to determine study eligibility (protocol section 5.16). All patients will have a repeat peritonoscopy to evaluate for tumor transduction (day 14) or response to treatment (day 35) and to evaluate for persistence of vector in the abdominal cavity (protocol section 4.19). At least two patients from each of the first two dose levels will undergo peritonoscopy on day 14 to document tumor cell transduction. Select patients may be asked to undergo additional peritonoscopies if they cannot be followed with CT scan to evaluate response. The inconsistencies in the protocol with regard to peritoneal washings have been clarified. Peritoneal washings will be performed on the day of screening peritonoscopy, and on protocol days 3, 7, 14, and on day 35. We revised Protocol section 5.24 to "Additional instillation of 1-2 liters of plasmalyte and withdrawal of 20 to 100 ml samples of fluid will be done on days 3, 7, 14, and 35 after the initial instillation of VPC to determine how long the injected cells remain viable in the patient." The catheter will be removed on day 35. The samples obtained by washings will be evaluated by light microscopy and cultured for persistent VPC, to isolate and evaluate for the presence of inflammatory cells, and to study tumor cells for LTKOSN.2 gene transfer.

2. Response to comment by Dr. Zallen. Dr. Zallen stated that the consent form should be modified to state that the treatment is unlikely to be beneficial and to include a discussion of the dose escalation. The revised consent form is attached with all changes in bold type.

3. Response to points discussed in the letter from Dr. Rother of Alexion Pharmaceuticals. Dr. Rother's letter discusses the effect of serum inactivation on VPC and retroviruses. We, as well as others, have been well aware of these effects on retroviruses for some time. Dr. Rother and his colleagues at Alexion pharmaceuticals have found some interesting information that a significant part of this rejection is related to an epitope, α-galactosyl, which is found on the cells of most mammalian species including Mus musculus, but not on the cells of old world primates, apes, or humans. This glycosylation difference has been tightly linked to hyperacute rejection induced by serum complement activated by α galactosyl antibodies. Interestingly this epitope has been reported to be present on aged human erythrocytes [1]. This has been noted despite the presence of two frameshift mutations in the human homologue of the α(1,3) galactosyl transferase gene [2]. Another group noted that range of αgal specificity varies with blood group since persons with type B or AB blood express the galα1-3(Fucα1-2)Galβ-4GlcNAc-R which is self antigen for these individuals [3]. The model of hyperacute rejection that prevents xenograft survival is typically a vascularized xenograft directly exposed to blood serum [4, 5]. These models are significantly different than the implantation of VPC into the peritoneal cavity where no direct exposure of the xenogeneic cells to serum typically would occur. Dr. Rother accurately notes that the available data in old

world primates with NIH3T3 based VPC injected into the brain showed an apparent resistance to hyperacute rejection. Two plausible explanations for the observation were suggested by Dr. Rother, reduced levels of complement and/or reduced levels of α(1,3)galactosyl antibodies in the CNS.

We elected to conduct a series of *in vitro* experiments to test the effect of peritoneal fluid on vector producer cells (VPC). In the first experiment, peritoneal fluid from a woman with ovarian carcinoma was incubated with LTKOSN.2 VPC for 24 to 67 hrs at various concentrations. In all cases, 1 x 10^6 cells were suspended in the appropriate concentration of peritoneal fluid mixed with RPMI with 10% fetal calf serum (R10) and then seeded into 25 cm^2 flasks (in duplicate). Cells were counted from one flask after 24 hrs and another flask after 67 hrs of exposure to various peritoneal fluid concentrations. Cells harvested after 67 hours of exposure from the 50% and 90% groups were then titered and found to have titers of 9.8 x 10^5 cfu/ml and 9.9 x 10^5 cfu/ml respectively. These titers are equivalent to freshly cultured LTKOSN.2 VPC. Interestingly, in concentrations of 75% or less peritoneal fluid, LTKOSN.2 VPC were able to proliferate. The plating efficiency of the cells in 100% peritoneal fluid was low.

Table 1. Cells remaining after exposure to various concentrations of peritoneal fluid mixed with R10 media.

Percent Peritoneal Fluid (mixed with R10)	Cell Number 24 hrs after Plating in Peritoneal Fluid	Cell Number 67 hrs after Plating in Peritoneal Fluid
10%	2.2 x 10^6	4.5 x 10^6
25%	1.9 x 10^6	4.3 x 10^6
50%	1.5 x 10^6	13 x 10^6
75%	9.0 x 10^5	1.2 x 10^6
90%	6.9 x 10^5	1.1 x 10^6
100%	7.5 x 10^4	3 x 10^4

In the second experiment, we tested additional peritoneal fluid samples. Sample #1 was from a patient with a benign ovarian cyst and sample #2 was from a patient with adenocarcinoma of the colon. We resuspended the cells in peritoneal fluid to resemble the *in vivo* human situation. LTKOSN.2 VPC (2X10^6 cells) were resuspended in 1 ml of 100%, 90%, or 50% peritoneal fluid diluted with R10, seeded into 6 well plates, and incubated for 5 hrs at 37°C. All unattached cells were transferred into a new 6-well plate and these unattached cells were counted by trypan blue dye exclusion.

Table 2. Effect of various concentrations f human periton al fluid on VPC.

Peritoneal fluid sample	Number of Unattached cells after 5 hrs of Treatment with Peritoneal Fluid (out of 2 x 10^6 cells)		
	100% Peritoneal Fluid	90% Peritoneal Fluid	50% Peritoneal Fluid
#1	1.5 x10^6 cells	1.2 x10^6 cells	0.65 x10^6 cells
	25% plating eff.	40% plating eff.	68% plating eff.
	0% viable	0% viable	50% viable
#2	1.7 x10^6 cells	1.0 x10^6 cells	0.3 x10^6 cells
	15% plating eff.	50% plating eff.	85% plating eff.
	0% viable	80% viable	65% viable

Table 2 notes the number of unattached cells after 5 hrs. We analyzed the population of cells that *did not* attach by trypan blue exclusion as a measure of toxicity induced by the peritoneal fluid. These are expressed in table 2 as percent viable cells. Interestingly, we plated cells from samples showing no viability by trypan blue exclusion and they still grew colonies of viable VPC. Cells that were able to attach and survive the exposure were considered viable and were next tested to see if they remained active.

We then determined the vector titer of the remaining (attached) cells on the plates after exposure to peritoneal fluid. The plates exposed to peritoneal fluid samples (100%, 90%, and 50%) had the peritoneal fluid removed and 2 mls of R10 were added to each well. After 48 hrs of incubation all wells were confluent. Media was replaced with fresh R10. After 24 hrs of incubation, a standard titration assay was performed. IGROV ovarian cancer cells were incubated with supernates from each well of the 6-well plate for 24 hrs, and then the medium was replaced with R10 containing 1 mg/ml of G418 and incubated for 7 days at 37°C, 5% CO_2. Titration plates were washed with HBSS and stained with methylene blue and the colonies were counted. The results are shown in table 3.

Table 3. Titer of LTKOSN.2 VPC after exposure to peritoneal fluid for 5 hrs.

	Percent Peritoneal Fluid in Well: cfu/ml after exposure to peritoneal fluid			
Peritoneal fluid (Percent mixed with R10)	100%	90%	50%	None (control)
Sample #1	6.0 x 10^5 cfu/ml	6.0 x 10^5 cfu/ml	7.3 x 10^5 cfu/ml	8.9 x 10^5 cfu/ml
Sample #2	4.2 x 10^5 cfu/ml	5.2 x 10^5 cfu/ml	4.7 x 10^5 cfu/ml	5.5 x 10^5 cfu/ml

These results demonstrate that despite exposure to peritoneal fluid the samples still maintained their titer, on the same order of magnitude as the control cells not exposed to peritoneal fluid.

Overall these results indicate that some toxicity of VPC will occur on exposure to peritoneal fluid. In our protocol the VPC will be suspended in 2 liters of plasmalyte that will greatly dilute these effects compared to the undiluted peritoneal fluid noted above. We also compared these results to toxicity induced by human serum and found that human serum causes a substantial destruction of VPC in culture. This finding is in agreement with the results reported by Dr. Rother. Peritoneal sample #1 induced much less destruction than that induced by human serum.

In conclusion, the work cited by Dr. Rother deals with hyperacute rejection induced by serum against xenogeneic cells and tissues. Our results suggest that these effects are substantially less with peritoneal fluid. The key unknown factor will be whether or not the VPC are able to survive long enough to induce a clinically significant amount of tumor cell transduction by LTKOSN.2 *in vivo*. Our clinical protocol should be able to obtain data to answer this question. We have added the following exclusion to Section 4.2 Exclusion criteria, "Patients estimated to have greater than 2 liters of ascites or tense ascites at the time of intraperitoneal infusion are not eligible."

4. One further modification we wish to make is to eliminate the lowest dose from the dose escalation schema. After discussing our dose escalation strategy with the FDA, we feel that the lowest dose planned could be dropped. We would ask permission to start the dose escalation at $1X10^6$ cells/kg.

5 Response to comment by Dr. Wivel. Your letter of March 17, 1995, correctly notes that ovarian cancer lesions can sometimes consist of tumor cells nested in fibrotic nodules. We have demonstrated that fibroblasts *in vitro* can actually provide excellent bystander effect for ovarian cancer cells. This implies that the transduction of fibroblasts within a fibrotic tumor nodule might actually be of benefit and contribute to the antitumor effect of this type of suicide gene therapy.

1. Galili, U., *et al., Man, Apes, and Old World Monkeys Differ from Other Mammals in the Expression of α-Galactosyl Epitopes on Nucleated Cells.* J. of Biol. Chem., 1988. **263**: p. 17755-17762.
2. Larsen, R.D., *et al., Frameshift and Nonsense Mutations in a human Genomic Sequence Homologous to a Murine UDP-Gal:β-D-Gal(1,4)-D-GlcNac α(1,3)-Galactosyltransferase cDNA.* J. of Biol. Chem., 1990. **265**: p. 7055-7061.
3. Galili, U., *Evolution and pathophysiology of the human natural anti-y-galactosyl IgG / antibody.* Springer Semin. Immunopathol., 1993. **15**: p. 155-171.

HUMAN GENE THERAPY
RESEARCH INSTITUTE
AN IOWA HEALTH SYSTEM AFFILIATE

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Una S. Ryan, Ph.D.
Vice President of Research and
Chief Scientific Officer
T-Cell Sciences
115 Fourth Ave
Needham, MA 02194
(617) 621-1400
(617) 433-3191FAX

Dear Dr. Ryan,

Thank you for your time today on the phone. As you suggested, I have included a brief experimental schema for an *in vitro* experiment to determine if sCR1 can block the complement mediated lysis of xenogeneic murine vector producer cells (based on NIH3T3 fibroblasts). Xenogeneic murine vector producer cells are destroyed by human complement mediated lysis against the α1,3-galactosyl epitope that is absent on cells from old world primates and humans.

I also will send to you our RAC approved protocol for the treatment of refractory or recurrent intraperitoneal ovarian carcinoma. In the peritoneal cavity, we feel the vector producer cells have a chance of surviving long enough to permit gene transfer. This general strategy would also be attempted for intrahepatic liver tumors. However, since intrahepatic lesions are in contact directly with blood, we anticipate that complement mediated destruction of xenogeneic cells would occur more readily under these conditions. This might not allow time for effective gene transfer into tumor cells.

We appreciate your consideration of this study. As you stated, if these results were promising, we could discuss possibilities for *in vivo* experiments.

Sincerely,

Charles J. Link Jr., M.D.
Associate Director

EXHIBIT
D

FORM B

006

Treatment Lot ID: LTKOSN.1

Product Lot #: PL #21

Number of vials: 11

Date of Infusion:

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	10	10	10	T.S.
2	Vol of Cell Suspension	100µl	100µl	100µl	T.S.
3	Volume of Vital Dye	900µl	900µl	900µl	T.S.
4	Total Vol of Cell Suspension	22µl	22µl	22µl	T.S.
5	# Viable cells in 4 quadrants	292	301	324	T.S.
6	# Non-viable cells in 4 quads	100	98	102	T.S.
7	Viable cells/ml = # viable:4xKx10^4 (K=dilution)	7.3×10^6	7.5×10^6	8.1×10^6	T.S.
8	Average Viable cells/ml	7.6×10^6 cells/µl			T.S.
9	Total # viable cells= (Step 8 x Step 4)	1.7×10^9			T.S.
10	% Viability = # viable cells ÷ (# non-viable + # viable)	74%	75%	76%	T.S.
11	Average Viability	75%			T.S.

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: MCE

EXHIBIT
E

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: 11,21,22,23

Number of vials: 68

Date of Infusion:

PL #22
Viability before freezing 85% (ODT)
after " — " 78%

PL #23

(patient viab after freezi 92%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	50	50	50	TS
2	Vol of Cell Suspension	100µl	100µl	100µl	TS
3	Volume of Vital Dye	4.9ml	4.9ml	4.9ml	TS
4	Total Vol of Cell Suspension	754ml	754ml	754ml	TS
5	# Viable cells in 4 quadrants	88	80	64	TS
6	# Non-viable cells in 4 quads	66	ND	ND	
7	Viable cells/ml = # viable:4xKx10^4 (K=dilution)	1.1×10^7	1.0×10^7	0.9×10^7	TS
8	Average Viable cells/ml	1.0×10^7			T.S.
9	Total # viable cells= (Step 8 x Step 4)	$\frac{1.0 \times 10^7}{4} \times 754 = 754 \times 10^9$			T.S.
10	% Viability = # viable cells ÷ (# non-viable + # viable)	ND	ND	ND	T.S
11	Average Viability	75%			

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: not

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: 11, 21, 22, 23

Number of vials: 68

Date of Infusion:

PL #22
Viability before freezing 85%
after " " 78%

PL #23

patient viab after freezi 92%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	50	50	50	T S
2	Vol of Cell Suspension	100µl	100µl	100µl	TS
3	Volume of Vital Dye	4.9ml	4.9ml	4.9ml	TS
4	Total Vol of Cell Suspension	754ml	754ml	754ml	TS
5	# Viable cells in 4 quadrants	88	80	64	TS
6	# Non-viable cells in 4 quads	66	ND	ND	
7	Viable cells/ml = # viable:4xKx10^4 (K=dilution)	1.1×10^7	1.0×10^7	0.9×10^7	T.S
8	Average Viable cells/ml	1.0×10^7			T.S.
9	Total # viable cells= (Step 8 x Step 4)	$\approx 1.0 \times 10^7 \times 754 = 754 \times 10^9$			T.S.
10	% Viability = # viable cells ÷ (# non-viable + # viable)	ND	ND	ND	T.S
11	Average Viability	75%			

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: noe

FORM B

PL#24 Harvest viab after freezing 99%

Treatment Lot ID: LTKOSN.1

Product Lot #: PL#24 and PL#29

Number of vials: PL#24=32vials, PL#29=10vials

Date of Infusion:

PL#29 - harvest viab. after freezing 92.5%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	10	10	10	TS
2	Vol of Cell Suspension	100µl	100µl	100µl	TS
3	Volume of Vital Dye	900µl	900µl	900µl	TS
4	Total Vol of Cell Suspension	640ml	640ml	640ml	TS
5	# Viable cells in 4 quadrants	400	392	380	TS
6	# Non-viable cells in 4 quads	112	130	120	TS
7	Viable cells/ml = # viable:4xKx10^4 (K=dilution)	10×10^6/ml	9.8×10^6/µl	9.5×10^6/µl	TS
8	Average Viable cells/ml	9.8×10^6/ml			TS
9	Total # viable cells= (Step 8 x Step 4)	6.3×10^9			TS
10	% Viability = # viable cells ÷ (# non-viable + # viable)	78%	75%	76%	TS
11	Average Viability	76%			TS

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: MG

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: PL#24 and PL#29

Number of vials: PL#24=32vials, PL#29 =10vials

Date of Infusion:

PL#24 harvest viab after freezing 98% 08

PL#29 - harvest viab. after freezing 72% 2.05

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	10	10	10	TS
2	Vol of Cell Suspension	100µl	100µl	100µl	TS
3	Volume of Vital Dye	900µl	900µl	900µl	TS
4	Total Vol of Cell Suspension	640ml	640ml	640ml	TS
5	# Viable cells in 4 quadrants	400	392	380	TS
6	# Non-viable cells in 4 quads	112	130	120	TS
7	Viable cells/ml = # viable:4xKx10^4 (K=dilution)	10×10^6/ml	9.8×10^6/µl	9.5×10^6/µl	TS
8	Average Viable cells/ml	9.8×10^6/ml			TS
9	Total # viable cells= (Step 8 x Step 4)	6.3×10^9			TS
10	% Viability = # viable cells ÷ (# non-viable + # viable)	78%	75%	76%	TS
11	Average Viability	76%			TS

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: MG

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: 18, 29, 30, 31

Number of vials: 78

Date of Infusion:

PL#18 harvest viab. after freezing = 78%

PL#29 harvest viab. after freezing = 72.5%

PL#30 harvest viab. after freezing 71%

PL#31 3-10-97 harvest viab. after freezing 91%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	10	10	10	T.S.
2	Vol of Cell Suspension	640	640	640	T.S.
3	Volume of Vital Dye	0.9ml	0.9ml	0.9ml	T.S.
4	Total Vol of Cell Suspension	0.1ml	0.1ml	0.1ml	T.S.
5	# Viable cells in 4 quadrants T.S.	93	104	118	T.S.
6	# Non-viable cells in 4 quads T.S.	31	26	24 T.S.	T.S.
7	Viable cells/ml = # viable:4xKx10^4 (K=dilution)	9.3×10^6	1.04×10^7	9.4×10^6	T.S.
8	Average Viable cells/ml	9.7×10^6			T.S
9	Total # viable cells= (Step 8 x Step 4)	6.2×10^9			T.S
10	% Viability = # viable cells ÷ (# non-viable + # viable)	75%	80%	80%	T.S.
11	Average Viability	77.5%			T.S.

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: EH

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: 18, 29, 30, 31

Number of vials: 78

Date of Infusion:

PL#18 harvest viab. after freezing = 78%

PL#29 harvest viab. after freezing – 72.5%

PL#30 viab. after freez 71%

PL#3 varie viab. after freezing 91%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	10	10	10	T.S.
2	Vol of Cell Suspension	640	640	640	T.S.
3	Volume of Vital Dye	0.9ml	0.9ml	0.9ml	T.S.
4	Total Vol of Cell Suspension	0.1ml	0.1ml	0.1ml	T.S.
5	# Viable cells in 4 quadrants T.S.	93	104	118	T.S.
6	# Non-viable cells in 4 quads T.S.	31	26	24	T.S.
7	Viable cells/ml = # viable:$4xKx10^4$ (K=dilution)	$9.3x10^6$	$1.04x10^7$	$9.4x10^6$	T.S.
8	Average Viable cells/ml	9.7×10^6			T.S
9	Total # viable cells= (Step 8 x Step 4)	6.2×10^9			T.S
10	% Viability = # viable cells ÷ (# non-viable + # viable)	75%	80%	80%	T.S.
11	Average Viability	77.5%			T.S.

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: Elt

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: Combined PL# 26 (26 vials) + PL# 32 (20 vials)

Number of vials: 46

Date of Infusion:

PL# 26 Viab. after harvest freezing 88.5%

PL# 32 harvest viab. after freeze 72%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	100	100	100	TS
2	Vol of Cell Suspension	100 µl	100 µl	100 µl	TS
3	Volume of Vital Dye	900 µl	900 µl	900 µl	TS
4	Total Vol of Cell Suspension	160 ml	100 ml	100 ml	TS
5	# Viable cells in 4 quadrants	80	71	78	TS
6	# Non-viable cells in 4 quads	32	27	34	TS
7	Viable cells/ml = # viable x K x 10^4 (K=dilution)	8×10^7/ml	7.1×10^7/ml	7.8×10^7/ml	TS
8	Average Viable cells/ml	7.6×10^7/ml			TS
9	Total # viable cells= (Step 8 x Step 4)	7.6×10^9			TS.
10	% Viability = # viable cells ÷ (# non-viable + # viable)	71%	72%	70%	TS
11	Average Viability	71%			TS.

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: MGE

FORM B

Treatment Lot ID: LTKOSN.1

Product Lot #: combined PL# 26 (26 vials) + PL# 32 (20 vials)

Number of vials: 46

Date of Infusion:

PL# 26 harvest viab. after freezing 88.5%

PL# 32 harvest viab. after freeze 72%

I. Total Cell Number Calculations

Step #	Calculations (volumes in ml)	Count 1	Count 2	Count 3	Verified by:
1	Dilution Factor	100	100	100	TS
2	Vol of Cell Suspension	100 µl	100 µl	100 µl	TS
3	Volume of Vital Dye	900 µl	900 µl	900 µl	TS
4	Total Vol of Cell Suspension	160 ml	100 ml	100 ml	TS
5	# Viable cells in 4 quadrants	80	71	78	TS
6	# Non-viable cells in 4 quads	32	27	34	TS
7	Viable cells/ml = # viable x K x 10^4 (K=dilution)	8×10^7/ml	7.1×10^7/ml	7.8×10^7/ml	TS
8	Average Viable cells/ml	7.6×10^7 /ml			TS
9	Total # viable cells= (Step 8 x Step 4)	7.6×10^9			TS.
10	% Viability = # viable cells ÷ (# non-viable + # viable)	71%	72%	70%	TS
11	Average Viability	71%			TS.

II. Gram Stain Reading:
A "pass" is no Gram positive or Gram negative colonies observed in 10 microscopic fields (100 x oil).

Results (circle one): **PASS** **FAIL**

Reading performed by: MGL

HGTRI 0101 - Ovarian Trial

Final Report

Polymerase Chain Reaction (PCR) Analysis for Gene Transfer of the Herpes Simplex-Thymidine Kinase (HStk) Gene

Test Article ID:

Patient number: HGTRI 0101:001
Sample type: tumor / peritoneal wash

Authorized Representative: Tatiana Seregina, Ph.D.

EXHIBIT
F

CONCLUSION

Patient number HGTRI 0101:001 **was found to be negative for HStk gene transfer on days** -1, 3, 7, 14, 35 **of the protocol.**

STUDY INFORMATION

Test Article:	Peritoneal washes and/or tumor biopsies obtained on days: -1, 3, 7, 14, 35 and -1, 14, 35, respectively.
Testing Facility:	Testing Group of the Clinical Applications Laboratory, Human Gene Therapy Research Institute, 1415 Woodland Avenue, Suite 218 Des Moines, IA 50309
Schedule:	
Initiation Date:	
Completion Date:	
Research Associate:	Jeffrey McDermott
Archives:	All raw data, records, protocols, and a copy of the report will be maintained by the testing facility at the above address.
Positive Control:	1) Genomic DNA isolated from cell mixes containing LTKOSN.1 and NIH3T3.nv. The range used will be 1:2000 ($5x10^{-4}$) and 1:10,000 ($1x10^{-4}$) of LTKOSN.1 to NIH3T3.nv, respectively. Alternatively, genomic DNA isolated from cell mixes containing retrovirally transduced A375.HStk and A375.nv. The ranges will be similar as above. 2) A know concentration of purified plasmid pLTKOSN.63, which contains the HStk gene.

	3) Use PCR to amplify GAPDH as an internal control (see SOP # CAL.T021).
Negative Control:	1) A sample containing no DNA to ensure no contaminates are in the PCR reagents. 2) Genomic DNA from NIH:OVCAR-3.nv cells, human adenocarcinoma ovarian cells.

I. OBJECTIVE

The study objective is to determine whether gene transfer of the HStk gene in the patient's tissues occurred after the infusion of the retrovirus producing cells line, LTKOSN.1.

II. PROCEDURES

A. Sample Preparation

A sample of the test article was prepared according to SOP # CAL.T018.

B. Methods

A sample of patient number HGTRT 0101 : 001 was PCR amplified for HStk gene according to CAL.T020. Additional screening involving Southern Blot Hybridization employed CAL.T022, CAL.T023, and CAL.T024.

III. RESULTS

1. The results of the PCR were recorded on Form PCR-B (a copy of the form is included).
2. Patient number HGTRT 0101 : 001 was found to be negative when tested for the presence of HStk using PCR amplification.

IV. APPROVAL

This study was performed in compliance with the principles of the U.S. FDA Good Laboratory Practice regulations (21 CFR 58).

Jeffrey P. McDermott — Date
Research Associate

Form PCR-B. Results of Polymerase Chain Reaction for Herpes Simplex-Thymidin Kinas .

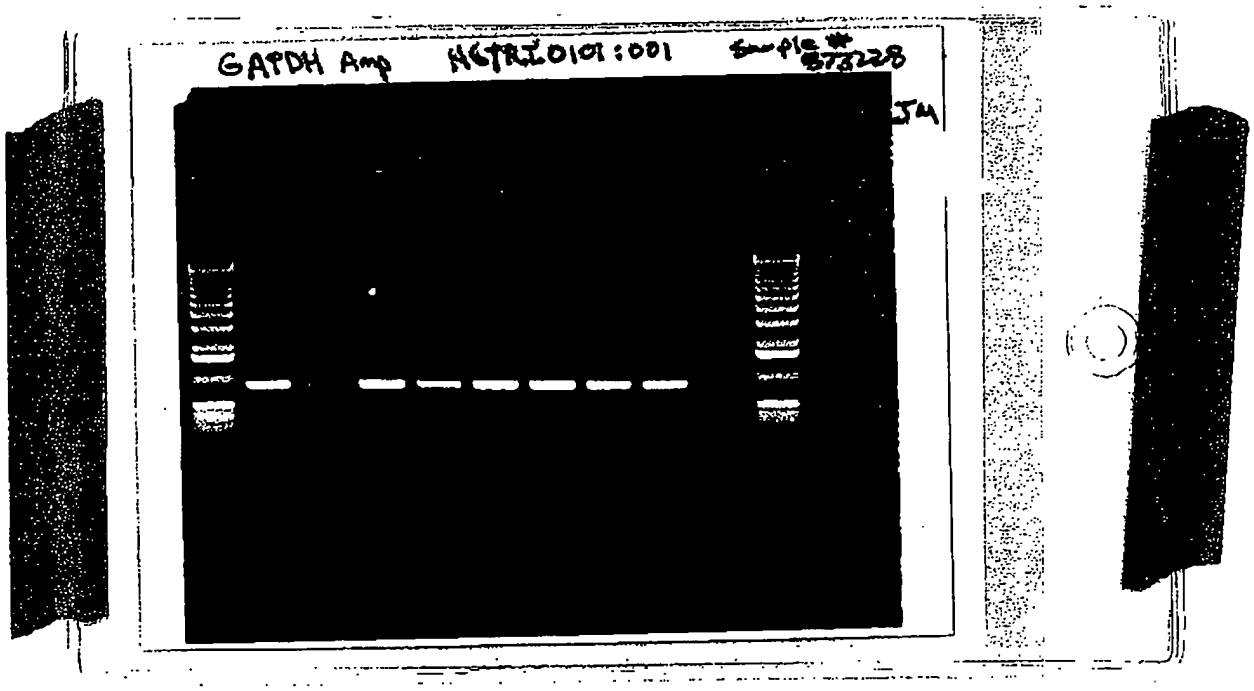
RESEARCH ASSOCIATE: Jeffrey P. McDermott

PATIENT NUMBER: H6TRI0101:001

DATE:

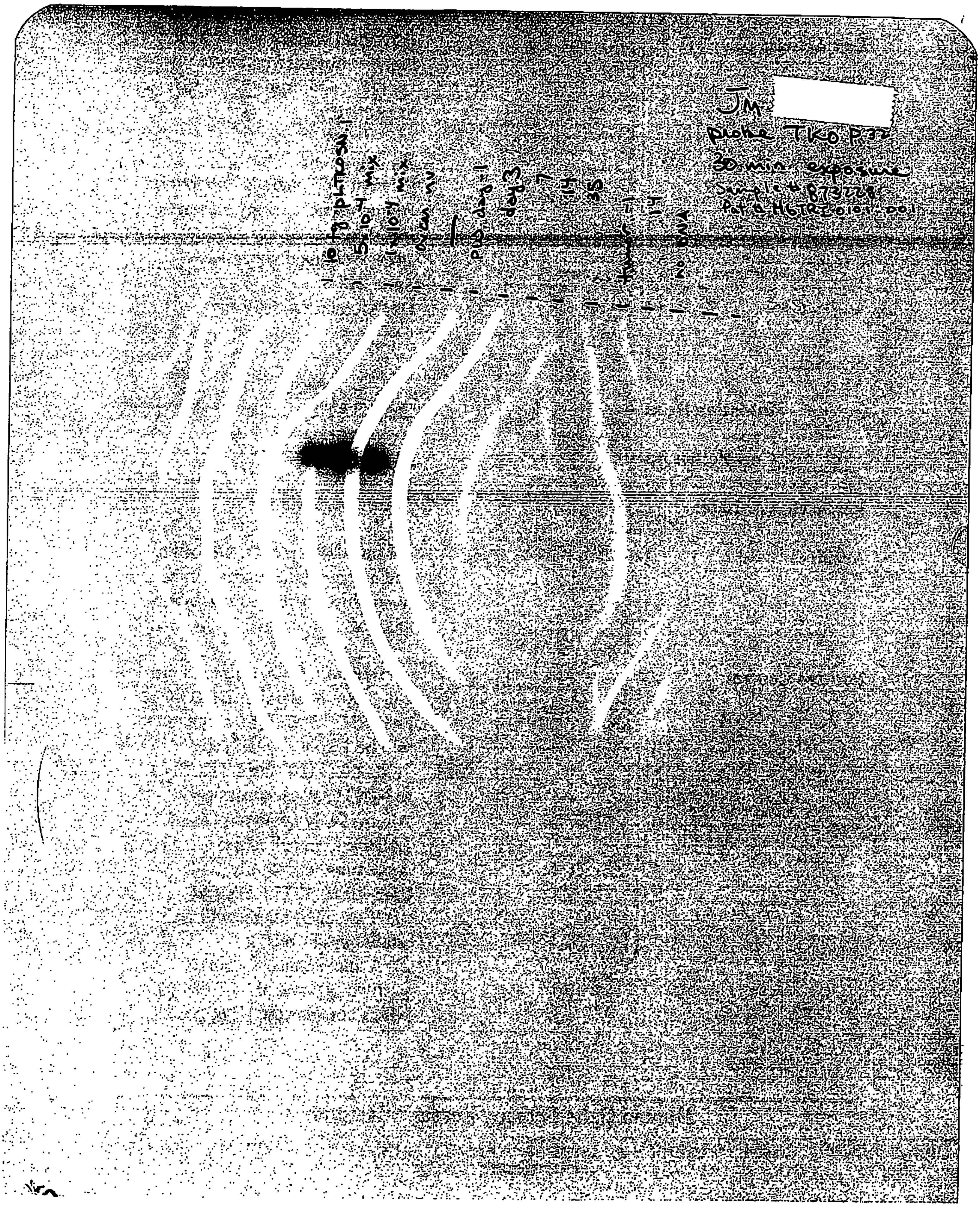
RESULTS:

PCR Amplification of GAPDH Gene Sequence



PCR Amplification of Herpes Simplex-thymidine kinase Gene Sequence

attached


JM
probe TKO P32
30 min exposure
Sample # 873228
Pat # H6TRI 0101-001
day 3
7
14
35
no DNA

Final Report

Polymerase Chain Reaction (PCR) Analysis for Replication Competent Retrovirus (RCR).

Test Article ID: Patient number: HGTRI 0101 : 001

Authorized Representative: Tatiana Seregina, Ph.D.

CONCLUSION

Patient number HGTRI 0101 : 001 **was found to be negative when tested for the presence of RCR using PCR amplification.**

STUDY INFORMATION

Test Article: Genomic DNA samples from whole blood drawn on days 0,1,7,14,21, and 28.

Testing Facility: Testing Group of the Clinical Applications Laboratory, Human Gene Therapy Research Institute, 1415 Woodland Avenue, Suite 218 Des Moines, IA 50309

Schedule:

Initiation Date:

Completion Date:

Research Associate: Jeffrey McDermott

Archives: All raw data, records, protocols, and a copy of the report will be maintained by the testing facility at the above address.

Positive Control:

1) Genomic DNA isolated from cell mixes containing LTKOSN.1 and NIH3T3.nv. The range used will be 1:2000 ($5x10^{-4}$) and 1:10,000 ($1x10^{-4}$) of LTKOSN.1 to NIH3T3.nv, respectively.

2) A know concentration of purified plasmid pPAM3, which contains the MMLV env gene.

3) Use PCR to amplify GAPDH as an internal control (see SOP # CAL.T021).

Negative Control:

1) A sample containg no DNA to ensure no contaminates are in the PCR reagents.

2) Genomic DNA from TALL.nv cells, human cytotoxic T-lymphocytes.

I. OBJECTIVE

The study objective is to determine whether replication competent retroviruses are present in the patient's peripheral blood cells (PBC) as determined by the amplification of the MMLV env gene through PCR.

II. PROCEDURES

A. Sample Preparation

A sample of the test article was prepared according to SOP # CAL.T018.

B. Methods

A sample of Patient number HGTRI 0101.001 was PCR amplified for MMLV env gene according to CAL.T019. Additional screening involving Slot Blot Hybridization employed CAL.T022, CAL.T023, and CAL.T024.

III. RESULTS

1. The results of the PCR were recorded on Form PCR-A (a copy of the form is included).

2. Patient number HGTRI 0101.001 was found to be negative when tested for the presence of RCR using PCR amplification.

IV. APPROVAL

This study was performed in compliance with the principles of the U.S. FDA Good Laboratory Practice regulations (21 CFR 58).

Jeffrey P. McDermott
Research Associate

Date

Form PCR-A. Results of Polymerase Chain Reaction for Replication Competent Retroviruses.

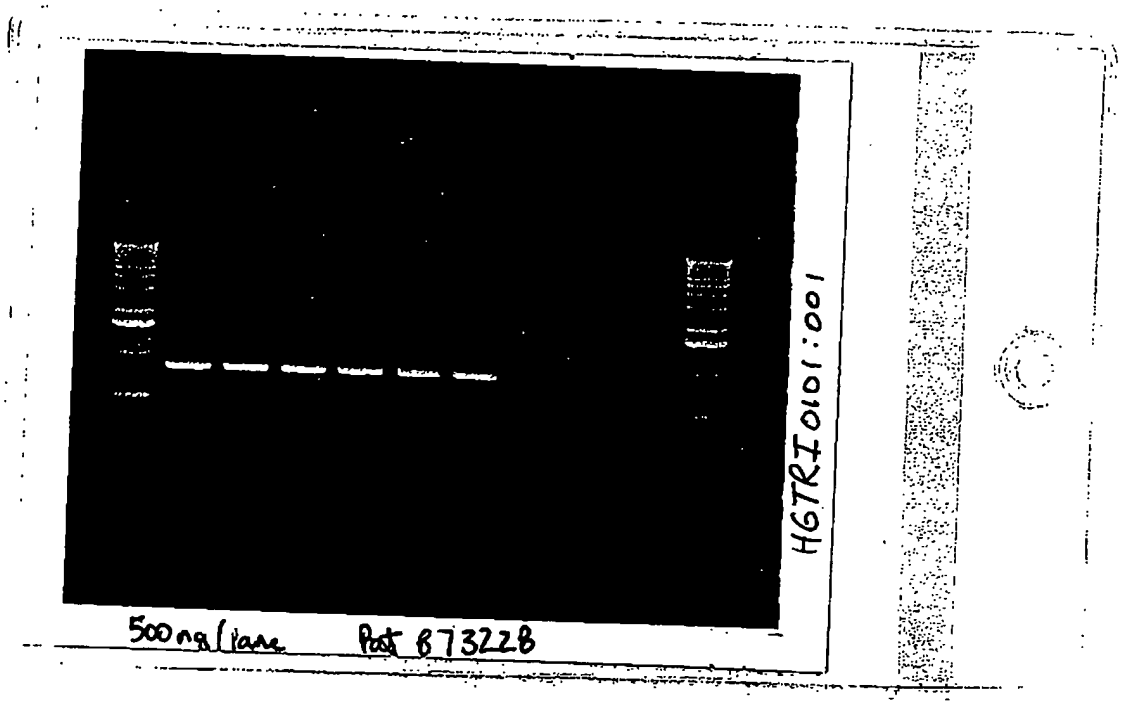
RESEARCH ASSOCIATE: Jeffrey P. McDermott

PATIENT NUMBER: HGTRI 0101:001

DATE:

RESULTS:

PCR Amplification of GAPDH Gene Sequence



PCR Amplification of MMLV Envelope Gene Sequence

See attached folder

HGTRI 0101 - Ovarian Trial

Final Report

Polymerase Chain Reaction (PCR) Analysis for Gene Transfer of the Herpes Simplex-Thymidine Kinase (HStk) Gene

Test Article ID:

Patient number: HGTRI 0101 : 002
Sample type: tumor / peritoneal wash

Authorized Representative: Tatiana Seregina, Ph.D.

CONCLUSION

Patient number HGTRI 0101 : 002 **was found to be negative for HStk gene transfer on days** -1, 3, 7, 14, 35 **of the protocol.**

STUDY INFORMATION

Test Article:	Peritoneal washes and/or tumor biopsies obtained on days: -1, 3, 7, 14, 35 and -1, 14, 35, respectively.
Testing Facility:	Testing Group of the Clinical Applications Laboratory, Human Gene Therapy Research Institute, 1415 Woodland Avenue, Suite 218 Des Moines, IA 50309
Schedule:	
Initiation Date:	
Completion Date:	
Research Associate:	Jeffrey McDermott
Archives:	All raw data, records, protocols, and a copy of the report will be maintained by the testing facility at the above address.
Positive Control:	1) Genomic DNA isolated from cell mixes containing LTKOSN.1 and NIH3T3.nv. The range used will be 1:2000 ($5x10^{-4}$) and 1:10,000 ($1x10^{-4}$) of LTKOSN.1 to NIH3T3.nv, respectively. Alternatively, genomic DNA isolated from cell mixes containing retrovirally transduced A375.HStk and A375.nv. The ranges will be similar as above. 2) A know concentration of purified plasmid pLTKOSN.63, which contains the HStk gene.

3) Use PCR to amplify GAPDH as an internal control (see SOP # CAL.T021).

Negative Control:

1) A sample containing no DNA to ensure no contaminates are in the PCR reagents.
2) Genomic DNA from NIH:OVCAR-3.nv cells, human adenocarcinoma ovarian cells.

I. OBJECTIVE

The study objective is to determine whether gene transfer of the HStk gene in the patient's tissues occurred after the infusion of the retrovirus producing cells line, LTKOSN.1.

II. PROCEDURES

A. Sample Preparation

A sample of the test article was prepared according to SOP # CAL.T018.

B. Methods

A sample of patient number HGTRI 0101 : 002 was PCR amplified for HStk gene according to CAL.T020. Additional screening involving Southern Blot Hybridization employed CAL.T022, CAL.T023, and CAL.T024.

III. RESULTS

1. The results of the PCR were recorded on Form PCR-B (a copy of the form is included).
2. Patient number HGTRI 0101 : 002 was found to be negative when tested for the presence of HStk using PCR amplification.

IV. APPROVAL

This study was performed in compliance with the principles of the U.S. FDA Good Laboratory Practice regulations (21 CFR 58).

Jeffrey P. McDermott
Research Associate

Date

Form PCR-B. Results of Polymerase Chain Reaction for Herpes Simpl x-Thymidine Kinase.

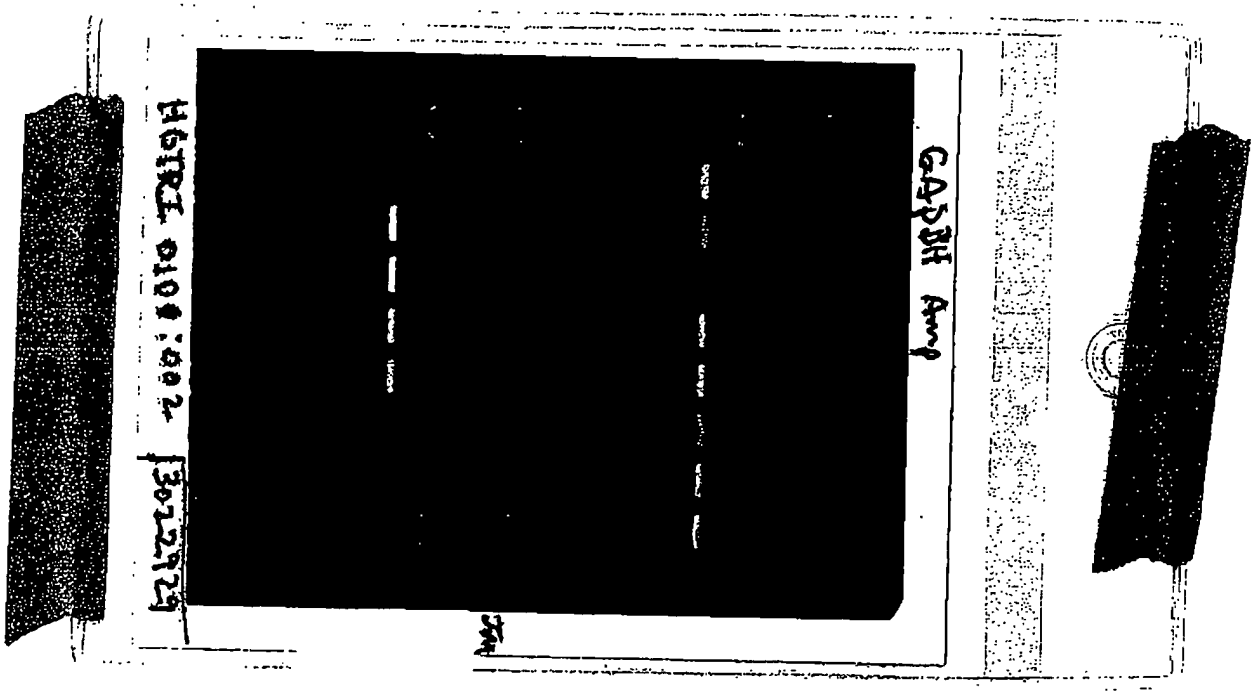
RESEARCH ASSOCIATE: Jeffrey P. McDermott

PATIENT NUMBER: HGTRI 0101 : 002

DATE:

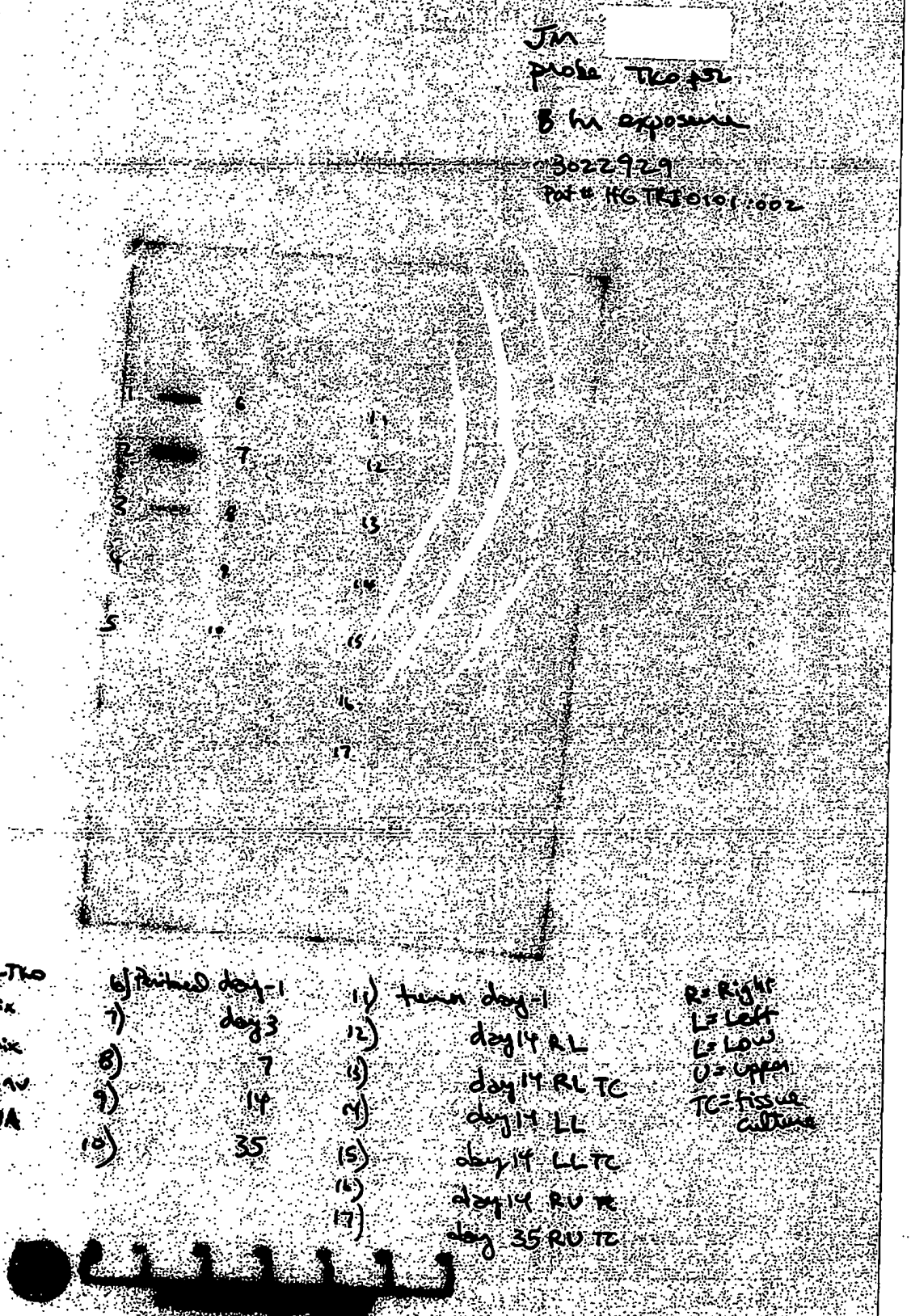
RESULTS:

PCR Amplification of GAPDH Gene Sequence



PCR Amplification of Herpes Simplex-thymidine kinase Gene Sequence

on back


JM
probe TLC #32
8 hr exposure
c3022929
Pat # HG TRJ0101.002
1) 10fg pLTR0
2) 5x10⁻⁴ mix
3) 1x10⁻⁴ mix
4) Ovcar-3
5) No DNA
6) Patient day-1
7) day3
8) 7
9) 14
10) 35
11) tumor day-1
12) day14 RL
13) day14 RL TC
14) day14 LL
15) day14 LL TC
16) day14 RU TC
17) day 35 RU TC
R = Right
L = Left
L = Low
U = Upper
TC = tissue culture

Final Report

Polymerase Chain Reaction (PCR) Analysis for Replication Competent Retrovirus (RCR).

Test Article ID: Patient number: HGTRI 0101 : 002

Authorized Representative: Tatiana Seregina, Ph.D.

CONCLUSION

Patient number HGTRI 0101 : 002 **was found to be negative when tested for the presence of RCR using PCR amplification.**

STUDY INFORMATION

Test Article: Genomic DNA samples from whole blood drawn on days 0,1,7,14,21, and 28.

Testing Facility: Testing Group of the Clinical Applications Laboratory, Human Gene Therapy Research Institute, 1415 Woodland Avenue, Suite 218 Des Moines, IA 50309

Schedule:

Initiation Date:

Completion Date:

Research Associate: Jeffrey McDermott

Archives: All raw data, records, protocols, and a copy of the report will be maintained by the testing facility at the above address.

Positive Control:

1) Genomic DNA isolated from cell mixes containing LTKOSN.1 and NIH3T3.nv. The range used will be 1:2000 ($5x10^{-4}$) and 1:10,000 ($1x10^{-4}$) of LTKOSN.1 to NIH3T3.nv, respectively.

2) A know concentration of purified plasmid pPAM3, which contains the MMLV env gene.

3) Use PCR to amplify GAPDH as an internal control (see SOP # CAL.T021).

Negative Control:	1) A sample containg no DNA to ensure no contaminates are in the PCR reagents. 2) Genomic DNA from TALL.nv cells, human cytotoxic T-lymphocytes.

I. OBJECTIVE

The study objective is to determine whether replication competent retroviruses are present in the patient's peripheral blood cells (PBC) as determined by the amplification of the MMLV env gene through PCR.

II. PROCEDURES

A. Sample Preparation

A sample of the test article was prepared according to SOP # CAL.T018.

B. Methods

A sample of Patient number HGTRI 0101.002 was PCR amplified for MMLV env gene according to CAL.T019. Additional screening involving Slot Blot Hybridization employed CAL.T022, CAL.T023, and CAL.T024.

III. RESULTS

1. The results of the PCR were recorded on Form PCR-A (a copy of the form is included).

2. Patient number HGTRI 0101.002 was found to be negative when tested for the presence of RCR using PCR amplification.

IV. APPROVAL

This study was performed in compliance with the principles of the U.S. FDA Good Laboratory Practice regulations (21 CFR 58).

Jeffrey P. McDermott
Research Associate

Date

Form PCR-A. Results of Polymerase Chain R action for Replication Comp tent Retroviruses.

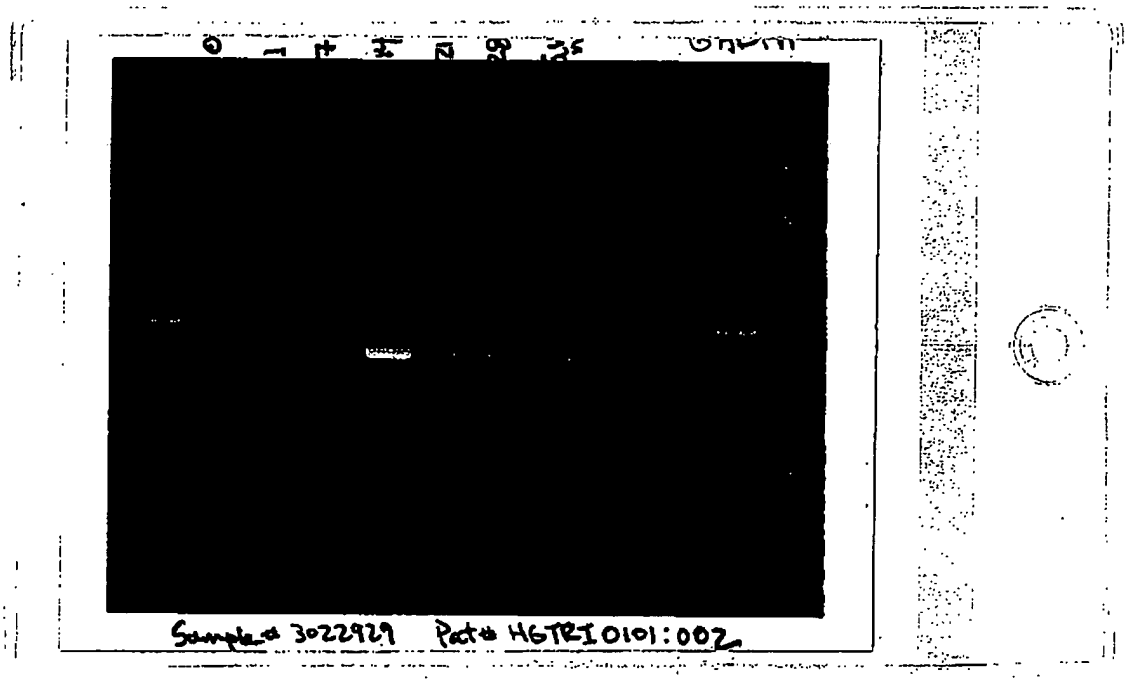
RESEARCH ASSOCIATE: Jeffrey P. McDermott

PATIENT NUMBER: HGTRI 0101 ;002

DATE:

RESULTS:

PCR Amplification of GAPDH Gene Sequence



PCR Amplification of MMLV Envelope Gene Sequence

KODAK SAFETY FILM ARO

KODAK SAFETY FILM ARO

HGTRI 0101 - Ovarian Trial

Final Report

Polymerase Chain Reaction (PCR) Analysis for the Herpes Simplex-Thymidine Kinase (HStk) Gene Sequence in Peripheral Blood Lymphocytes (PBL'S)

Test Article ID:

Patient number: HGTRI 0101.'004
Sample type: Genomic DNA for PBL's

Authorized Representative: Tatiana Seregina, Ph.D.

CONCLUSION

Patient number HGTRI 0101.'004 **was found to be negative for HStk gene sequence in PBL's on days** 0, 14 & 28 **of the protocol.**

STUDY INFORMATION

Test Article: Genomic DNA was isolated from PBL's.

Testing Facility: Testing Group of the
Clinical Applications Laboratory,
Human Gene Therapy Research Institute,
1415 Woodland Avenue,
Suite 218
Des Moines, IA 50309

Schedule:

Initiation Date:

Completion Date:

Research Associate: Martin Edelman

Archives: All raw data, records, protocols, and a copy of the report will be maintained by the testing facility at the above address.

Positive Control: 1) Genomic DNA isolated from cell mixes containing A375.TKO and A375.nv. The range used will be $1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$ and $1x10^{-5}$ of A375.TKO to A375.nv, respectively.

2) A know concentration of purified plasmid pLTKOSN.63, which contains the HStk gene.
3) Use PCR to amplify GAPDH as an internal control (see SOP # CAL.T021).

Negative Control: 1) A sample containing no DNA to ensure no contaminates are in the PCR reagents.
2) Genomic DNA from A375.nv cells.

I. OBJECTIVE

The study objective is to determine the presence of the HStk gene in the patient's PBL's after the infusion of the retrovirus producing cells line, LTKOSN.1.

II. PROCEDURES

A. Sample Preparation

A sample of the test article was prepared according to SOP # CAL.T018.

B. Methods

A sample of patient number H67RI0101:004 was PCR amplified for HStk gene according to CAL.T020. Additional screening involving Slot Blot Hybridization employed CAL.T022, CAL.T023, and CAL.T024.

III. RESULTS

1. The results of the PCR were recorded on Form PCR-B (a copy of the form is included).
2. Patient number H67RI0101:004 was found to be negative when tested for the presence of HStk using PCR amplification.

IV. APPROVAL

This study was performed in compliance with the principles of the U.S. FDA Good Laboratory Practice regulations (21 CFR 58).

Research Associate Date

Form PCR-B. Results of Polymerase Chain Reaction for Herpes Simplex-Thymidine Kinase.

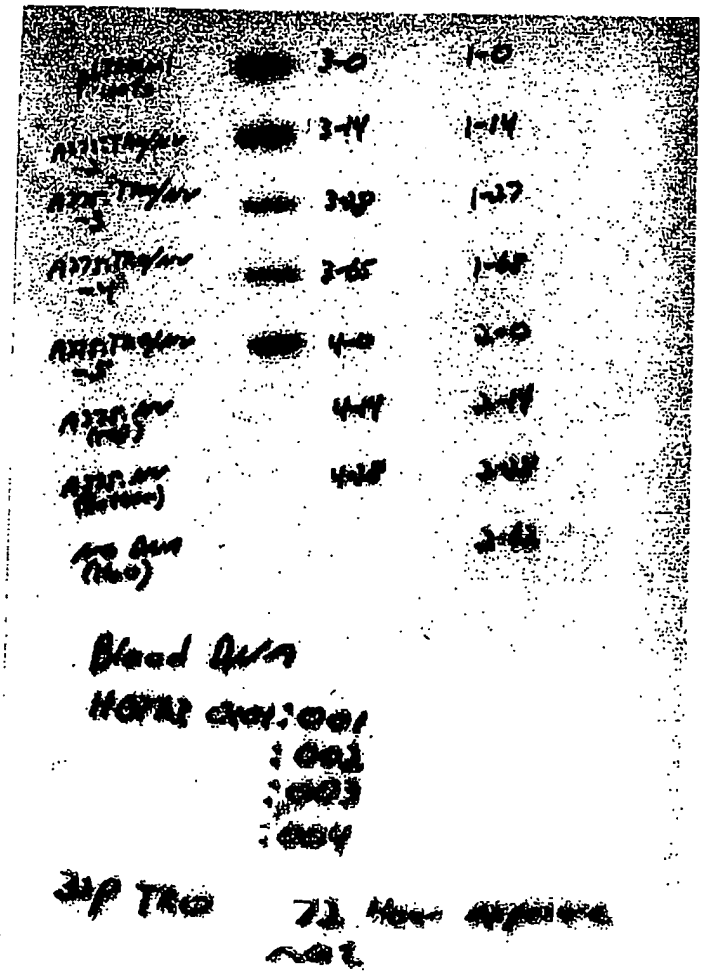
RESEARCH ASSOCIATE: Martin Edleman

PATIENT NUMBER: HGTRI0101:004

DATE:

RESULTS:

PCR Amplification of Herpes Simplex-thymidine kinase Gene Sequence



See Lab book # 0208 P# 33 for original

ALLEGHENY UNIVERSITY OF THE HEALTH SCIENCES
Department of Microbiology & Immunology
2900 Queen Lane
Philadelphia, PA 19129
Phone: (215) 991-8350
FAX: (215) 848-2271

pt #1-5

DATE:

TO: Dr. C. Link

FAX #: 515-241-8788

PAGES: 3
(Including this cover sheet)

FROM: Dr. Uri Galili

COMMENTS: Enclosed are the ELISA data. The starting dilution is 1:25. The all have somewhat low anti-Gal activity. However #5 displays an approximately 16-20 fold increase in anti-Gal titer, whereas #4 shows 4 fold increase. The increase in #5 (and possibly #4) are probably the result of immune response to the mouse cells as you have predicted.

Fax TRANSMISSION

EXHIBIT
G

MULTISKAN PLUS VERSION 2.03

ABS. MEAS.
FILTER 492

ABS.

04:36:22

Elisa for Human sera.
α-Gal-BSA 10 µg/ml.
Anti-Human IgG (HRP) 1:1000

Sera Dilution	1	2	3	4	5	6	7	8	9	10	11	12
A	1.592	1.722	1.843	1.630	1.687	1.815	1.783	2*012	1.932	1.991	1.162	1.197
B	0.951	0.959	0.911	0.781	0.679	0.824	0.789	0.851	0.911	1.203	0.746	0.653
C	0.529	0.586	0.487	0.503	0.454	0.542	0.496	0.528	0.603	0.735	0.558	0.652
D	0.329	0.365	0.287	0.303	0.399	0.334	0.293	0.338	0.339	0.438	0.327	0.416
E	0.260	0.245	0.205	0.186	0.157	0.207	0.185	0.192	0.221	0.280	0.209	0.242
F	0.128	0.135	0.120	0.140	0.110	0.135	0.116	0.135	0.139	0.158	0.187	0.221
G	0.090	0.097	0.083	0.086	0.082	0.093	0.105	0.166	0.090	0.108	0.095	0.147
H	0.065	0.068	0.069	0.078	0.079	0.074	0.071	0.070	0.106	0.076	0.070	0.094
2fold dilutions	1-1	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	1-10	2-1	2-2
days	0	1	7	14	15	21	27	68	96	138	0	1

pt #1

MULTISKAN PLUS VERSION 2.03

ABS. MEAS.
FILTER 492

ABS.

04:36:52

Elisa for Human Sera.
α-Gal-BSA 10 µg/ml.
Anti-Human IgG HRP 1:1000

Sera Dilution	1	2	3	4	5	6	7	8	9	10	11	12
A	1.607	1.327	1.375	1.414	1.560	1.234	1.363	0.862	1.576	1.522	1.475	1.706
B	0.556	0.838	0.919	0.955	1.044	0.824	0.564	0.641	0.540	0.568	0.455	0.579
C	0.346	0.598	0.718	0.655	0.829	0.576	0.335	0.433	0.351	0.362	0.275	0.345
D	0.225	0.398	0.459	0.428	0.539	0.405	0.224	0.303	0.219	0.231	0.181	0.216
E	0.160	0.260	0.304	0.281	0.362	0.279	0.148	0.204	0.140	0.146	0.118	0.152
F	0.097	0.156	0.185	0.175	0.226	0.181	0.103	0.138	0.102	0.100	0.087	0.099
G	0.075	0.117	0.151	0.120	0.164	0.120	0.076	0.097	0.078	0.210	0.070	0.075
H	0.069	0.091	0.106	0.093	0.112	0.090	0.069	0.078	0.068	0.067	0.072	0.094
	2-3	2-4	2-5	2-6	2-7	2-8	3-1	3-2	3-3	3-4	3-5	3-6
	7	14	21	28	62	114	0	1	7	14	21	28

pt#2 pt#3

pt#1 - no change
#2 - " "
#3 - two

MULTISKAN PLUS VERSION 2.03

ABS. MEAS.
FILTER 492

ABS.

04:38:26

Elisa for Human sera
α-Gal-BSA 1 ug/ml
Anti-Human IgG HRP (1:1000)

Sera 1:25	1	2	3	4	5	6	7	8	9	10	11	12	
B	1.035	1.391	0.709	0.780	0.710	1.527	1.805	1.779	1.761	2*201	2*210	2*529	1:25
C	0.390	0.545	0.285	0.339	0.278	0.621	0.736	0.770	0.819	1.207	1.136	1.177	1:50
D	0.238	0.348	0.188	0.211	0.176	0.406	0.393	0.477	0.563	0.853	0.711	0.834	1:100
E	0.162	0.224	0.135	0.142	0.127	0.256	0.260	0.319	0.340	0.561	0.527	0.568	1:200
F	0.108	0.141	0.094	0.100	0.091	0.158	0.168	0.203	0.208	0.317	0.308	0.344	1:400
G	0.085	0.106	0.074	0.079	0.079	0.109	0.115	0.132	0.133	0.194	0.183	0.199	1:800
H	0.075	0.084	0.066	0.067	0.066	0.085	0.107	0.127	0.092	0.132	0.115	0.133	1:1600
2-fold	0.106	0.114	0.079	0.082	0.078	0.083	0.077	0.074	0.073	0.106	0.131	0.118	1:3200
	3-1	3-2	4-1	4-2	4-3	4-4	4-5	4-6	4-7	5-1	5-2	5-3	
	65	143	0	1	7	14	16	21	28	2	4	7	pre GCV

#3 pt #4 pt #5

MULTISKAN PLUS VERSION 2.03

ABS. MEAS.
FILTER 492

ABS.

04:38:42

Sera 1:25	1	2	3	4	5	6	7	8	9	10	11	12
B	1:25 2*412	2*584	2*247	1.990	1.846	0.054	0.000	0.000	0.000	0.000	0.000	0.000
C	1:50 2*721	2*835	2*672	2*703	2*780	0.057	0.000	0.000	0.000	0.000	0.000	0.000
D	1:100 2*516	2*516	2*685	2*647	2*679	0.077	0.000	0.000	0.000	0.000	0.000	0.000
E	1:200 2*045	1.933	2*472	2*619	2*430	0.065	0.002	0.000	0.000	0.000	0.000	0.000
F	1:400 1.283	1.420	1.857	2*118	2*144	0.059	0.003	0.000	0.000	0.000	0.000	0.000
G	1:800 0.907	0.889	1.263	1.481	1.382	0.063	0.000	0.000	0.001	0.002	0.000	0.000
H	1:1600 0.602	0.574	0.850	0.972	0.929	0.056	0.000	0.000	0.000	0.000	0.000	0.000
	1:3200 0.432	0.440	0.534	0.671	0.696	0.064	0.000	0.000	0.001	0.000	0.000	0.000
	5-4	5-5	5-6	5-7	5-8							
	14 pre GCV	14 post GCV	21	28 post GCV	28 pre GCV							

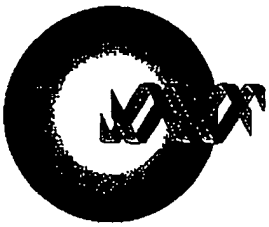
pt #5

pt #3 was ...
pt #4 = 4 fold ... after GCV
pt #5 = 16 fold ... after GCV

Volume 5, Number 6

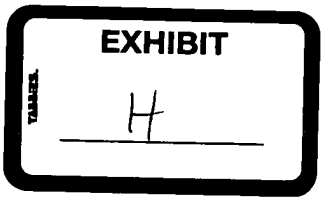
A CONFERENCE SUPPLEMENT TO

CANCER GENE THERAPY



Gene Therapy of Cancer VII

San Diego, California



STOCKTON

microenvironment to one that is immunostimulatory and have broad implications as an anticancer chemoimmunotherapy.

O-49

Herpes simplex virus thymidine kinase (HSV-TK) murine vector producer cell (VPC) xenografts in women with recurrent ovarian cancer

CJ Link, DW Moorman, J Levy, L Tennant, M Edleman, J McDermott, U Galili, and T Seregina

Human Gene Therapy Research Institute, John Stoddard Cancer Center, Iowa Methodist Medical Center, Des Moines, Iowa.

We are conducting a phase I trial of patients with recurrent ovarian cancer using the xenotransplantation of murine retroviral vector producer cells (VPCs) for *in vivo* transfer of the herpes simplex virus thymidine kinase (HSV-tk) gene. All patients entering the trial had recurrent or refractory epithelial ovarian or fallopian tube cancer and had failed prior therapy with paclitaxel and either cisplatin or carboplatin. Patients received an intraperitoneal infusion of LTKOSN.1 VPCs in doses from 50×10^6 to 7×10^9 cells (1×10^6 to 1×10^8 cells/kg) followed 2 weeks later by ganciclovir (GCV) treatment. After the xenotransplant, patients developed low grade fever and mild to moderate abdominal pain for 2–5 days. A VPC-specific DNA sequence (retroviral amphotropic *env*) could be detected by polymerase chain reaction at days 3 and/or 7 after infusion at the middle and highest dosage levels ($0.1–1 \times 10^8$ cells/kg). Viable VPCs were recovered on day 3 from one patient. VPCs were not detected by polymerase chain reaction in any patient after day 7, suggesting their rapid clearance from the peritoneal cavity. Tumors that were biopsied on day 14 prior to the administration of GCV showed the presence of the HSV-tk gene. Four of eight patients demonstrated some evidence of an antitumor response. No complete responses have been observed to date. Of note, one patient evaluated by laparoscopy prior to GCV infusion demonstrated a significant resolution of malignant ascites. This observation implied a role for xenotransplant rejection in the observed antitumor response. We hypothesized that a major hyperacute rejection antigen present on murine cells, α(1,3)galactosyl epitope (α-gal), might be responsible for the finding. Therefore, we measured the anti-α-gal Ab titer both before and after the xenotransplant. Patients infused with $\geq 0.1 \times 10^8$ cells/kg demonstrated a 4–16-fold increase in anti-α-gal titer over the following 4 weeks. These data suggest that the process of xenotransplant rejection of murine VPCs may induce the immune destruction of human cancer. A phase II trial of LTKOSN.1 VPCs for ovarian cancer is planned.

O-50

Mechanisms of action of the synergistic effects of interferon-α (IFN-α) and ganciclovir (GCV) on tumor cell killing in the herpes simplex virus thymidine kinase (HSV-TK) system

Katharine A Whartenby,[1] James W Darnowski,[1] Scott M Freeman,[2] and Paul Calabresi[1]

[1]Brown University and Rhode Island Hospital, Providence, Rhode Island; and [2]Schering-Plough Corporation, Kenilworth, New Jersey.

We have demonstrated that interferon-α (IFN-α) synergistically enhances the ganciclovir (GCV)-mediated killing of human ovarian and prostate tumor cells genetically modified to express the herpes simplex virus thymidine kinase (HSV-TK) gene and the bystander killing of nearby unmodified tumor cells, both *in vivo* and *in vitro*. Studies to elucidate the mechanism of this activity are in progress. Initial studies assessed the effect of IFN-α on GCV incorporation into and clearance from cellular DNA and revealed that exposing either modified or parent cells to IFN-α for 24 hours did not affect these metabolic parameters. Parallel studies to assess the effect of IFN-α and GCV exposure on DNA repair also revealed that DNA repair processes in this model are not affected. However, the addition of GCV increased the percentage of cells in S phase, and the combination with IFN-α significantly enhanced the increase. We have recently isolated total cellular polymerases from HSV-TK$^+$ cells and observed that both IFN-α and GCV combine to dramatically inhibit polymerase activity; this result is consistent with the ability of these agents to delay progression through S phase.

Assessment of the effect of exposure to IFN-α and/or GCV on cellular metabolism are in progress. Preliminary studies have revealed that intracellular concentrations of NAD^+ were decreased following treatment with the two agents, suggesting that energy-dependent processes have been disrupted. Studies are ongoing to evaluate effects of IFN-α and GCV on concentrations of other intracellular nucleotide pools.

Supported by the TJ Martell Foundation and Rhode Island Hospital.

O-51

Development of targeted cytokine/suicide gene therapy for naturally occurring canine malignant tumors: A model for human cancer therapy?

DJ Argyle, L Nasir, C McGillivery, L Nicolson, and DE Onions

Molecular Therapeutics Unit, University of Glasgow Veterinary School, Glasgow, United Kingdom.

Naturally occurring cancer in the dog is of major significance in terms of animal health and welfare and, after correcting for lifespan, the canine population has the highest rate of malignant tumors among domestic species. The aim of this project is to develop targeted "suicide" and cytokine gene therapy for the treatment of neoplastic disease in the dog and to use this as a model for human cancer therapy.

Tumor Types and Targeting: Two tumors of particular interest to this group are canine malignant melanoma and canine osteosarcoma. To evaluate melanoma-specific expression, a plasmid-based reporter construct containing the CAT gene under the control of a human tyrosinase promoter (kindly provided by Dr. R. Vile, Imperial Cancer Research Fund, Hammersmith Hospital, London, U.K.) was used to transfect a variety of canine cell lines including a canine melanoma cell line. CAT levels were negligible in cell lines other than canine melanoma cells, suggesting that the human promoter is active in these cells and that expression is specific.

Using a combination of immunohistochemistry and DNA-sequencing techniques, we have demonstrated that p53 overexpression occurs in ~60% of osteosarcoma cases; a number of mutations associated with this tumor suppressor gene have been identified. Therefore, a targeted approach to osteosarcoma therapy could exploit p53 overexpression, and we are currently investigating potential strategies.

Cytokine Systems: Interleukin (IL)-12 and IL-18 are potent inducers of cell-mediated immunity and may act synergistically to promote an antitumorigenic immune response. We have cloned canine-specific IL-18 and IL-12 by reverse transcriptase polymerase chain reaction of RNA isolated from canine alveolar macrophages. Expression constructs have been generated, and the production of biologically active protein was confirmed in tissue culture using standard bioassays.

Conclusion: The aim of this project is to develop a gene-based therapy that combines tumor cell-specific killing with the promotion of antitumor immunity for maximal effect against tumor mass and micrometastases. We have established that melanoma-specific expression can be achieved in tissue culture and have cloned the canine cytokines IL-18 and IL-12. The next stage is to construct a targeted vector expressing a suicide gene from a melanoma-specific promoter, the effects of which may be enhanced by the coexpression of IL-12 and IL-18. Investigation of the *in vitro* and *in vivo* effects of this vector will represent a significant advance in targeted cancer gene therapy in the dog and could facilitate the development of novel therapies in human oncology.

O-52

Evaluation of plasmid pGL1-TNF-α in combination with radiation in a malignant brain tumor model

J Li, MA Andres, I Fodor, GA Nelson, JM Slater, and DS Gridley

Departments of Microbiology and Molecular Genetics and Radiation Medicine, Radiation Biology Program, Loma Linda University and Medical Center, Loma Linda, California.